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June 5, 2001

The Honorable Christine Todd Whitman
Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Comments on Cinnamyl Derivatives

Dear Administrator Whitman:

The following comments on the test plan for the cinnamyl derivatives are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than nine million Americans.

The Flavor and Fragrance High Production Volume Consortia have developed a well-constructed category and have presented robust summaries that adequately address each health endpoint of the SIDS battery and all but one ecotoxicity endpoint. Any further testing on animals would not contribute to the understanding of the toxicity of the cinnamyl derivatives. We recommend that no further testing on animals be conducted and that the proposed fish toxicity tests be deleted.

Chemical Category

This chemical category includes cinnamaldehyde, *alpha*-amylcinnamaldehyde, alpha-hexylcinnamaldehyde, and p-t-*alpha*-methylhydrocinnamaldehyde, which are unsubstituted or alkyl-substituted compounds with 3-phenyl-2-propenal or 3-phenylpropanol backbones. These substances are common household substances found in fragrance and food flavoring products. Cinnamaldehyde is the main component of cassia oil and Ceylon cinnamon bark oil. These compounds are structurally and toxicologically similar and therefore form a sensible category.

Available Information

The cinnamyl derivatives are essentially cinnamon oils and are well-understood, well-characterized chemicals that are used in food flavoring or fragrance products. No further animal testing on these substances is necessary, given their low toxicity and the available human data. Cinnamaldehyde, alpha-amylcinnamaldehyde, and alpha-hexylcinnamaldehyde are all labeled Generally Recognized as Safe

(GRAS) food additives by the Food and Drug Administration. The understanding of these common household substances go well beyond the crude SIDS battery. Metabolism (Smith 2000), toxicity (Kwon 1998, Stammati 1999, Verrier 1999), clinical (Johansen 1996, Prescott 2000), and occupational epidemiological studies (Meding 1993) of cinnamyl derivatives have been conducted with humans or *in vitro* with human cells. These chemicals have low toxicity, and the main human health concern is the sensitization or irritation that may be caused by exposure to cinnamon or related products. Scientists and occupational health professionals have a good handle on the mechanisms of allergic reactions to these substances. Any additional testing would not aid in the understanding or management of risks or adverse health effects associated with these substances.

Aquatic Toxicity

The proposed aquatic toxicity tests are unnecessary given the overall consistently low observed toxicity of these compounds, their low water solubility (<200 mg/L for all but one compound), and the availability of reliable nonanimal tests that would provide the information needed for these compounds for the HPV program. If any testing were to be conducted, certainly *in vitro* or QSAR methods should be used instead of the fish toxicity tests. Given the benign nature of these chemicals and the extensive understanding of aquatic microorganisms and *in vitro* test methods, any toxicity testing on fish is wholly inappropriate and unnecessary. *In vitro* tests with the protozoan *Tetrahymena* are frequently used as a measure of aquatic toxicity in ecological risk assessments (Larsen 1997). The biochemistry and physiology of *Tetrahymena* have been thoroughly investigated since the 1950s, and *Tetrahymena*, especially *T. pyriformis*, have been used for aquatic toxicity testing since the 1970s. Moreover, the genomics of the organism is currently being elucidated. The *T. pyriformis* population growth test is quick, easy, and cheap, and has incredible breadth (Schultz 1997). It allows the examination of a large number of independent organisms that possess features of both single eukaryotic cells and multicellular organisms. Studies at varying concentration levels can easily be repeated and many chemicals can be examined in a short period of time. Range-finding tests allow accurate approximation of both the highest concentration with no observed effect on population growth and the lowest concentration with total inhibition of cell replication. Fish toxicity tests are less economical, inhumane, slower, and more labor intensive.

The EPA has a massive database on the acute toxicity of more than 600 organic chemicals to fish called "Acute Toxicities of Organic Pollutants to Fathead Minnows (*Pimephales promelas*)." Comparisons of toxicity test results from the *in vitro* TETRATOX assay and the EPA's fish acute toxicity data have yielded good correlation between the two methods (Sinks 2001). Similarly, good correlation was observed between ciliate and guppy fish toxicity (Seward 2001). Moreover, where there is not good agreement, there is a logical rationale for this departure (Bearden and Schultz 1998). Evaluation of *in vitro* and *in vivo* aquatic toxicity data have allowed researchers like Schultz and colleagues to develop models to predict toxicity based on quantitative structure activity relationships, QSARs (Schultz 1999, Schultz and Cronin 1999, Niculescu 2000). Both the *in vitro* TETRATOX assay as well as QSARs provide more humane, efficient methods to predict aquatic toxicity at the screening level. We have requested a meeting with the EPA to discuss how to incorporate these alternative, nonanimal methods into the HPV program.

Thank you for the opportunity to comment, and I look forward to your response. I can be reached via telephone at 202-686-2210, ext. 302, or via e-mail at <ncardello@pcrm.org>. Correspondence should be sent to my attention at the following address: PCRM, 5100 Wisconsin Ave., Suite 400, Washington, DC 20016.

Sincerely,

Nicole Cardello, M.H.S.
Staff Scientist

cc: The Honorable Sherwood Boehlert
The Honorable Ken Calvert
The Honorable Jerry Costello
The Honorable Robert C. Smith

References

Bearden AP, Schultz TW. Comparison of *Tetrahymena* and *Pimephales* toxicity based on mechanism of action. SAR QSAR in Environmental Research 1998;9:127-53.

Johansen JD, Andersen KE, Rastogi SC, Menne T. Threshold responses in cinnamic-aldehyde-sensitive subjects: results and methodological aspects. Contact Dermatitis 1996;34(3):165-71.

Kwon BM, Lee SH, Choi SU, et al. Synthesis and *in vitro* cytotoxicity of cinnamaldehydes to human sold tumor cells. Arch Pharm Res 1998;21(2):147-52.

Meding B. Skin symptoms among workers in a spice factory. Contact Dermatitis 1993;29(4):202-5.

Niculescu SP, Kaiser LE, Schultz TW. Modeling the toxicity of chemicals to *Tetrahymnea pyriformis* using molecular fragment descriptors and probabilistic neural networks. Archives of Environmental Contamination and Toxicology 2000;39:289-98.

Prescott J, Swain-Campbell N. Responses to repeated oral irritation by capsicin, cinnamaldehyde, and ethanol in PROP tasters and non-tasters. Chem Senses 2000;25(3):239-46.

Schultz TW. Structure-toxicity relationships for benzenes evaluated *with Tetrahymena pyriformis*. Chemical Research in Toxicology 1999;12:1262-1267.

Schultz TW. Tetratox: *Tetrahymena pyriformis* population growth impairment endpoint–a surrogate for fish lethality. Toxicological Methods 1997;7:289-309.

Schultz TW, Cronin MTD. Response-surface analyses for toxicity to *Tetrahymena pyriformis*: reactive carbonyl-containing aliphatic chemicals. Journal of Chemical Information and Computer Sciences 1999;39:304-9.

Seward JR, Hamblen EL, Schultz TW. Regression comparison of *Tetrahymena pyriformis* and *Poecilia reticulata* toxicity. Chemosphere 2001; (in press).

Sinks GD, Schultz TW. Correlations of *Tetrahymena* and *Pimephales* toxicity: evaluation of 100 additional compounds. Environmental Toxicology and Chemistry 2001;20:917-21.

Smith CK, Moore CA, Elahi EN, Smart AT, Hotchkiss SA. Human skin absorption and
metabolism of the contact allergens, cinnamic aldehyde and cinnamic alcohol. Toxicol Appl Pharmacol 2000;163(3):189-99.

Stammati A, Bonsi P, Zucco F, Moezelaar R, Alakomi HL, Wright A. Toxicity of selected plant volatiles in microbial and mammalian short-term assays. Food Chem Toxicol 1999;37(8):813-23.

Verrier AC, Schmitt D, Staquet MJ. Fragrance and contact allergens in vitro modulate the HLA-DR and E-cadherin expression on human epidermal Langerhans cells. Int Arch Allergy Immunol 1999;120(1):56-62.